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Metal and proton toxicity to lake zooplankton: a chemical speciation based modelling approach

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1. Introduction

Current environmental regulations rarely require assessment of mixtures of chemicals. However, research on metal mixtures in the environment, and the development of efficient and economic science-based risk assessment approaches for mixtures, are vital for ensuring adequate environmental protection and for being prepared for future regulatory demands (Van Genderen et al., 2012). Several methods have been proposed to relate organism responses to the concentrations of mixtures of potentially toxic metals in the field. A pragmatic means of combining single-metal Environmental Quality Standards (EQS) for the field assessment of mixtures is through the use of Cumulative Criterion Units (Clements et al., 2008), calculated as the sum of the ratios of the concentration of each metal to its individual EQS. However, this only predicts an acceptable limit, not a concentration-response relationship, and as applied to date does not take full account of chemical speciation and bioavailability. The concept of multi-substance Potentially Affected Fractions has been developed, in which results from experiments with single toxicants are used to predict the proportion of species affected by a chemical mixture in the field, assuming either concentration or response additivity (De Zwart and Posthuma, 2005 and De Zwart et al., 2006). The Biotic Ligand Model has been applied in a meta-analysis study of metal mixtures, by assessing competition for binding at the biotic ligand by toxic metals (Zn, Cu, Cd), calcium and other major cations (Kamo and Nagai, 2008). A ‘saturation’ model, where toxicity is assumed to be a function of the amount of metal bound to a specific binding site on the organism, has also been tested for mixtures (e.g. Borgmann et al., 2004 and Norwood et al., 2007). The toxic unit approach, including incorporation of BLM modelling, has been applied to zooplankton communities of smelter-affected lakes in Sudbury, Canada (Khan et al., 2012). However, rather than using field biology data to define toxic effects, the majority of these approaches generally incorporate laboratory toxicity data and then use a derived model to make predictions of toxicity in field settings.

The chemical speciation code WHAM VI (Windermere Humic Aqueous Model; Model VI; Tipping, 1998;http://www.ceh.ac.uk/products/software/wham/) calculates the distribution of chemical species within waters, accounting for competition for binding sites on dissolved organic matter between protons and multiple cationic metals. Relationships between organism binding of metals and predictions of metal binding to humic acid (HA) using WHAM have been shown for bryophytes (Tipping et al., 2008) and stream macroinvertebrates (Stockdale et al., 2010). The WHAM- FTOX model (Stockdale et al., 2010), is based on this ability of

WHAM to yield results that are a proxy for metal binding to organisms. It is assumed that, at steady-state, the WHAM calculated bound metal and proton correlate with the “metabolically available” cations of the organisms, as defined by Rainbow (2002), i.e., variability in humic acid bound metals and protons will reflect the variability in the metabolically available metal and protons. The ability of WHAM (specifically the binding of metals and protons to HA) to predict the accumulation of metals by biota is supported by recent work showing correlation between measurements and modelling for several different taxa (Tipping et al., 2008, Stockdale et al., 2010 and Tipping and Lofts, 2013). WHAM-FTOX quantifies the combined toxic effects of protons and metal cations towards aquatic organisms through the toxicity function (F_{TOX}), a linear combination of the products of organism-bound cation and a toxic potency coefficient for each cation. Stockdale et al. (2010) parameterised WHAM-FTOX using the combined species richness of the macroinvertebrate groups Ephemeroptera, Plecoptera and Trichoptera (EPT) as the ecological indicator. In subsequent work Stockdale et al. (2014) used the EPT version of the model to compare temporal predictions of maximum macroinvertebrate species richness with field observations and found good agreement between observed and predicted recovery rates from acidification. The use of cation binding to HA as a proxy for accumulation by organisms, and consequent toxicity, has also been reported by Antunes et al. (2012) and Iwasaki et al. (2013).

The WHAM-F_{TOX} model is a promising tool relating the chemical speciation of natural waters to field biological responses. In order to increase the utility of the model for applications related to environmental monitoring and protection there is a need to establish relationships for additional biological taxa. Here, we apply WHAM- F_{TOX} to over 1000 observations of pelagic zooplankton species richness and associated water chemistry from surveys of 184 individual lakes from across Europe and North America, which have been studied with respect to either acidification as a result of atmospheric acid deposition or the impacts of metals from historical and continuing smelting operations in the Sudbury region of Canada (Keller et al., 2007). Pelagic zooplankton are good target organisms for this analysis as they have a constant exposure (including via food) to the medium (water) where chemistry is measured. Moreover, zooplankton populations have an important role in lake functioning, by affecting water clarity through grazing, and providing food for fish and predatory invertebrates (Makarewicz and Likens, 1979). The aims were (1) to obtain model parameters applicable to lake crustacean zooplankton species richness and (2) to assess model utility by comparing predictions with observations of temporal change, for cases where monitoring has been performed for a period of 10 years or more.

Although analysis of field data is attractive because it addresses toxicity directly within the given environment, a drawback is that species richness depends upon environmental factors other than cation toxicity. Such factors may be biotic (trophic status, competition, predation and food web structure), physical (temperature, lake area, stratification, suspended sediment), or chemical (presence of anionic toxicants such as arsenic, organic pollutants). Examples of these factors in the literature are provided by Keller and Conlon (1994; lake depth and area); Stemberger et al. (1996; temperature); Kratz et al. (1997; elevation); Jeppesen et al. (2000; productivity); Valois et al. (2010; predation); Nierzwicki-Bauer et al. (2010; predation). Both chemical toxicity and adverse unknown factors are assumed to reduce species numbers below a maximum that may be expected to be observed if all factors were at their optimum. As factors other than cation toxicity are not universally quantified across all surveys, we employed quantile regression, a statistical technique that provides a more complete view of possible causal relationships between variables in ecological processes than the simple use of linear response variables (Cade and Noon, 2003). This is achieved by fitting the data with a ‘roof’ that seeks to capture variations in zooplankton species richness that are due solely to chemical (cation) factors. This approach has previously been applied to assessing organism responses to metals in freshwaters, including (Stockdale et al., 2010 and Schmidt et al., 2012) and disregarding (Pacheco et al., 2005, Crane et al., 2007 and Linton et al., 2007) chemical speciation. The ‘roof’ fitted by quantile regression is in essence a prediction of the species numbers in the absence of adverse unknown factors but in the presence of varying degrees of chemical toxicity. This is under the assumption that the theoretical maximum has no spatial variability.

2. Methods

2.1. Data sources and data processing

We required datasets that included both water chemistry and zooplankton species richness for the same lakes. We collated data from several studies reported in peer reviewed literature from both time series and spatial studies. Detailed location descriptions, including number of lakes and the years over which sampling was undertaken, are included in Table 1. Data were obtained for the Bohemian Forest region, Czech Republic (Vrba et al., 2003); ranges of locations in Norway (the same sites and years as reported in Stockdale et al., 2014; zooplankton data previously reported in Walseng et al., 2006 and Skjelkvåle, 2010) and Sweden (Persson, 2008); the English Lake District region of the UK; Lake Plessa 107, formed as a result of lignite mining in Germany (Lessmann et al., 1999); lakes in the Adirondacks region of New York state, USA (Nierzwicki-Bauer et al., 2010); and two surveys of lakes in the Sudbury region of Ontario, Canada, one spatial (Valois et al., 2010) and one temporal (Khan et al., 2012). The Norwegian and Swedish sites are geographically spread across the respective countries but are not necessarily representative of either country. The Sudbury data include a number of sites that are known to have historical metal pollution, the majority of other sites are, or were, affected by acidification.

Crustacean zooplankton data were from summer collections and represent the total species richness (number of species; SR_{Czoo}) of the pelagic zone. Data are either from a single sampling period or are averaged over several sampling periods. When littoral species were found within the water column during sampling they were included in the value for species richness for all study regions except the Bohemian Forest lakes. The Supporting information provides details of sampling methodology for each region and species lists for each regional group of lakes.

Chemistry data were either means of multiple samples taken during the year (Bohemian Forest region, Czech Republic, Norway, Sweden and Adirondacks, USA), or from a single sample (Lake Plessa 107, English Lake District and Sudbury, Canada datasets). Details of the sampling and analysis methods are given in the site-specific references above, except for the English Lake District (Maberly et al., 2011 and Tipping et al., 1988). The 10th and 90th percentiles of chemical data are provided in the Supporting Information. Not all datasets included all of the required input data for trace metals and in these cases we used assumed ‘background’ values in the modelling. These assumed data are italicised in the Supporting information.

2.2. Chemical speciation

The WHAM model (http://www.ceh.ac.uk/products/software/wham/) calculates the equilibrium distributions of dissolved chemical species based on the following input parameters; temperature, pH, pCO_2, and the concentrations of DOC, major ions and trace metals. The model incorporates the Humic Ion-Binding Model VI (Tipping, 1998), which uses a structured formulation of discrete, chemically plausible, binding sites for protons, allowing the creation of regular arrays of bidentate and tridentate binding sites for metals.

Metal aquo ions (Al^{3+}, Fe^{3+}, etc.) and their first hydrolysis products ($AlOH^{2+}$, $FeOH^{2+}$, etc.) compete with each other, and with protons, for binding. The same intrinsic equilibrium constant is assumed to apply to the aquo ion and its first hydrolysis product (Tipping et al., 2008). In calculating aqueous chemical speciation, the concentrations of Na, Mg, K, Ca, Cl, NO_3 and SO_4 were assumed to represent dissolved components, as were concentrations of filterable trace metals (Ni, Cu, Zn, Cd and Pb). We used reactive Al for the calculations when it was available, but where filtered Al data were provided, the activity of Al was calculated from the measured total filtered concentration and from the generalised equation derived by Tipping (2005), with the lower of the two values being adopted. This avoided over-estimation of Al activity in filtrates containing colloidal forms of the element. The activity of Fe(III) was calculated from the empirical equation of Lofts et al. (2008). Concentrations of dissolved organic matter (DOM) were based on measured [DOC], assuming DOM to be 50% carbon and that 65% of it is active with respect to cation binding and can be represented by fulvic acid (Tipping et al., 2008). For example, for a DOC concentration of 5 mg L^{-1}corresponds to a fulvic acid concentration of 6.5 mg L^{-1} for modelling. For each speciation calculation, cation binding to humic acids (the proxy for accumulation by organisms) is computed, by assuming the presence of 10^{-6} gHA L^{-1}, which is sufficiently low not to affect the solution distribution of species (i.e. HA is not affecting the overall speciation, FA is present as the dominant DOM component).

2.3. The WHAM-F_{TOX} model

The combined toxicity of the cations is taken to be the sum of the products of (a) bound proton or metal cation (ν_i, mmol gHA^{-1}) and (b) a cation-specific toxicity coefficient (α_i) that characterises the relative potency of the individual cation. Thus we obtain a linear toxicity function;

$$F_{TOX}=\sum\alpha_i \cdot \nu_i \qquad (1)$$

Note that the values of ν_i are taken to be proportional, but not equal, to accumulation by the organism, since only relative binding is required. We describe the dataset with a lower threshold, $F_{TOX\text{-}LT}$, below which no toxic effects occur and an upper threshold, $F_{TOX\text{-}UT}$, above which all crustacean zooplankton species are absent. Thus we have the following conditions, in terms of species richness (SR_{Czoo}; Eqs. (2), (3) and (4)));

$$F_{TOX} \le F_{TOX\text{-}LT} \qquad SR_{Czoo} = SR_{Czoo\text{-}max} \qquad (2)$$

$F_{\text{TOX-LT}} < F_{\text{TOX}} < F_{\text{TOX-UT}}$ (3)

$$\text{SR}_{\text{Czoo}} = \text{SR}_{\text{Czoo-max}} - \left(\text{SR}_{\text{Czoo-max}} \times \frac{F_{\text{TOX}} - F_{\text{TOX-LT}}}{F_{\text{TOX-UT}} - F_{\text{TOX-LT}}}\right)$$

$F_{\text{TOX}} > F_{\text{TOX-UT}}$ $\text{SR}_{\text{Czoo}} = 0$ (4)

2.4. Data analysis

When examining field biological data, a direct correlation between predicted and actual species richness is expected only when modelled chemical behaviour is the sole factor that reduces species richness. To account for other unknown factors that also reduce richness, we used quantile regression. In this approach, quantiles of the response variable are employed, rather than the conditional mean (Cade and Noon, 2003). Quantiles were estimated with an optimisation function that minimises the sum of weighted absolute deviations, using the Solver function in Microsoft Excel©.

The use of a structured dataset, comprising individual subsets of spatially and/or temporally linked datasets, introduces the possibility of bias into the estimation of regression parameter variance. In conventional (least squares) regression, this would be alleviated by the use of a linear mixed modelling approach. In this work, however, the use of quantile regression minimises the risk of introducing bias in parameter variance due to the relaxation of the assumptions regarding the distribution of residuals about the regression line, therefore we have elected not to consider the data subset structure in parameterising the model.

We confirmed the significance of the contribution to F_{TOX} of proton and individual metals by performing *t*-tests. Uncertainties in the model parameters $\text{SR}_{\text{Czoo-MAX}}$, $F_{\text{TOX-UT}}$, $F_{\text{TOX-LT}}$, α_{Al}, α_{Ni}, α_{Cu} and α_{Zn} were estimated by bootstrapping, sampling the dataset with replacement 2000 times. The median, 15.9%ile and 84.1%ile of the distribution of each parameter were calculated; the range enclosed by these percentiles is a nonparametric equivalent of taking ±1 standard deviation of a normal distribution.

Regional Kendall testing (Helsel and Frans, 2006) was used to determine whether there were unidirectional trends over time and if a consistent trend occurred across an entire area at multiple locations. We analysed observed and modelled time series data of 10 years or more (Bohemian Forest, Norway, Adirondacks and Sudbury). Previous work compared temporal trends in predicted and observed macroinvertebrate species richness (Stockdale et al., 2014).

This work used WHAM-FTOX parameters derived from independent data (Stockdale et al., 2010) and data distribution across the prediction 'roof' was consistent with the independent fitting. Here we are performing temporal analysis for the same data as used for fitting.

3. Results and discussion

3.1. WHAM-F_{TOX} fitting to crustacean zooplankton data

Values of the amount of proton or metal bound to humic acid extracted from the WHAM model (Table 2) give an overall indication of the various cation chemical stressors within each of the survey areas. Values for protons are always above 1 mmol g^{-1} but reach over 4 for the extremely acid site Lake Plessa 107. Aluminium has a large range, from low values in circumneutral environments (e.g., English Lake District, <1 μ g L^{-1}), to high in many of the acidification affected regions (e.g., Lake Plessa 107, >1 mg L^{-1}). Trace metals are generally present at bound concentrations an order of magnitude or more lower that H^+ or Al. The ranges for all cations reveal the large inter-regional and geographical variability in the chemical measure.

All datasets were combined for fitting, in order to obtain global parameters (Table 3). Species richness was related to water chemistry by fitting the model to the 0.9th quantile, selected because higher quantiles may result in greater errors in fitted parameters (Cade and Noon, 2003). Toxicity coefficients (α_i), the F_{TOX}thresholds (F_{TOX-LT} and F_{TOX-UT}) and the maximum value for species richness ($SR_{CZOO-MAX}$) were optimised. Different starting values were applied to ensure that the correct optimisation minimum was reached. Provided that (a) the values were not initially set to zero, (b) F_{TOX-UT} was forced to exceed F_{TOX-LT} and (c) the initial estimate for $SR_{CZOO-MAX}$ was no higher than the maximum observed value of 16, the model always converged to give consistent values.

Values of the fitted toxicity coefficients (α_i) were statistically significant for Al, Ni, Cu and Zn, but not for either Cd or Pb. Toxic potencies increase in the order H^+ < Al < Cu < Zn < Ni, which is broadly similar to WHAM-F_{TOX} derived values for stream macroinvertebrates (Stockdale et al., 2010). However, α_{Cu} and α_{Zn} for the zooplankton (Table 3) are greater than the values of 3.50 and 2.69 respectively obtained for the macroinvertebrates, implying different sensitivities to trace metal toxicity between the two groups of organisms. However, we cannot rule out the possibility that physiological differences between taxa mean that using HA as a proxy for organism binding may not be equally appropriate for zooplankton and macroinvertebrates. The toxic contribution of each cation (metal or proton) depends on the cation's toxicity coefficient, combined with its bound concentration. Thus, a metal that exhibits weak binding but has a comparatively high toxicity coefficient can exert the same toxic effect as a strongly binding metal with a comparatively low toxicity coefficient. Thus, in the present study, the high value of α_{Ni} arises because the speciation model predicts weak binding of Ni by HA. The values of F_{TOX-LT} and F_{TOX-UT} for zooplankton (Table 3) are similar to those estimated for stream macroinvertebrates (2.33 ± 0.48 and 5.20 ± 0.65 respectively).

The “broken stick” line in Fig. 1 shows the modelled effects of water chemistry on species richness in terms of F_{TOX}. Points falling below the line indicate that additional un-modelled (i.e. non-chemical) factors have reduced species numbers. Overall, these effects are less marked when compared to previous findings for stream macroinvertebrates, suggesting that zooplankton diversity in lakes is subject to fewer or weaker non-chemical disturbances than macroinvertebrates in streams.

Plots of F_{TOX} against pH, with data points separated according to the contribution of trace metals (Ni, Cu and Zn) to F_{TOX}, show that toxicity due to trace metals is encountered mainly in the pH region 6 to 7.5 (Fig. 2), and these data largely reflect the temporally studied lakes at Sudbury. The most extreme case is Lake Plessa 107 which has high concentrations (>10 μmol L^{-1}) of both Ni and Zn. However, the very low pH of 2.3, and thus high competition for humic binding sites from protons, means that neither of these metals are calculated to be significantly bound by humic acid (the proxy for non-specific sites on the organism) and so neither contributes to F_{TOX}. In the absence of significant contributions from trace metals, F_{TOX} exceeds the lower threshold for all sites at acid pH, although the pH above which exceedance occurs varies with water chemistry (see below). The same effect was found for macroinvertebrates (Stockdale et al., 2010).

3.2. Results for individual datasets

3.2.1. Bohemian Forest lakes (Czech Republic)

The lakes included in this study were strongly acidified by acid deposition during the 1960s and 1970s (Vrba et al., 2003, Veselý et al., 1998 and Oulehle et al., 2013). The pH increased by between 0.5 and 0.9 units since 1984. Predicted and observed recovery in species richness was ~0.5 species per decade over the study period (Table 4). The constituent bound metals that make up the F_{TOX} value (mainly Al for this region) can partly explain the slow recovery rates for this region. Comparing data from 1984 to 2007‑9 (three year average), we predicted only a small decrease in F_{TOX} of between 0.13 and 0.19 for all lakes except Prášilské (decrease of 0.4). The contribution of v_H to F_{TOX} has decreased, but this has partly been offset by an increase in v_{Al}.

Regional Kendall tests (Table 4) show that recovery in crustacean zooplankton species richness was both predicted from chemistry and observed in the Bohemian Forest lakes. There is fair agreement between recovery trends predicted from maximum modelled species richness (0.52 species per decade) and observations (0.43 species per decade). For these sites there is a comparatively large gap between the modelled maximum species richness and the observed values (Fig. 3), which may be explained partly by the absence of littoral species from this dataset (numbering up to 6; see the Supporting information for details).

3.2.2. Norway

Regional Kendall testing (Table 4) shows actual recovery (0.7 species per decade) slightly higher than the predicted recovery of 0.5 species per decade. The discrepancy may be related to regional factors such as ongoing natural slow colonisation of isolated lakes, which would still occur if chemical conditions remained constant. Data from this survey show how such biogeographic differences between lakes (considered as un-modelled factors in our analysis) can additionally affect the species richness. Observations and model calculations of maximum species richness for the Norwegian lakes are shown in Fig. 4. Highland lakes all exhibited observed species richness significantly less than the maximum values predicted by the modelling. In contrast boreal and lowland sites exhibited some sites with close agreement. In addition to slow colonisation, the impact of dispersal barriers such as mountain ridges separating adjacent lowland areas may affect the species richness across some lakes within this study. We compared the mean difference in F_{TOX} between observations and modelling and lake altitude (Fig. 5). These results give further evidence that those lakes in the highland climate region show larger differences between modelled maximum values and observations. Additional analysis of this type demonstrates the potential for future statistical models to include additional drivers.

3.2.3. Sweden

The Swedish lakes differ from other acid lakes in that they had high dissolved organic matter concentrations (mean of 10 mg DOC L^{-1}), which altered the calculated binding of Al to humic acid (i.e. our proxy of organism binding), through chemical speciation. Differences from other lakes can be seen in Fig. 2 where these data have F_{TOX} values largely below the lower threshold, but at a much lower pH compared with other data, particularly over the pH range 6.0‑6.6. This shows the predicted protective effects that can be exhibited by DOM, as higher binding to fulvic acid (directly related to measured DOM in our modelling) results in lower binding to the humic acid (fixed concentration as the proxy for organism binding).

The data for Swedish lakes fall appreciably below the F_{TOX} prediction line, and to a greater degree than some other sets. This may be due to lower richness values caused by biogeographic differences, similar to the Norwegian lakes, as discussed above. Lower taxonomical resolution (many taxa identified only to genus level) may also be an explaination.

3.2.4. English Lake District (Cumbria, United Kingdom)

No toxic effects are predicted for these lakes and therefore it appears that non-chemical environmental influences constrain species richness below the maximum predicted by the model. Previous work has shown that variation in zooplankton species richness among these lakes is at least partly related to variations in productivity, as indicated by proxies such as concentrations of chlorophyll *a* and soluble reactive phosphorus (Thackeray, 2007). Enhanced lake productivity may reduce species richness due to correlated changes in resource quality, predation pressure and physico-chemical factors, e.g. oxygen concentrations (Dodson et al., 2000). The English Lake District case study therefore highlights a need for future work to evaluate the influence of metal toxicity on community structure, within the context of other measured environmental drivers.

3.2.5. Sudbury (Ontario, Canada)

The model predicts recovery of 1.6 species per decade, in good agreement with the observed value of 1.4 (Table 4). Recovery trends can be seen clearly for the individual lakes (Fig. 6). Recovery in species richness was more marked than in other regions owing to the combined effects of increased pH and DOC concentrations and decreasing concentrations of Ni, Cu, and Zn. Changes reflect both reductions in inputs and manipulative liming. Middle, Hannah and Lohi lakes were all limed in the 70s. Lohi Lake was limed in 1973, 1974 and 1975, but it re-acidified, before slowly naturally recovering, Hannah Lake was limed in the spring of 1975, after two years of monitoring. The pH of Middle and Hannah remained high because their watersheds were later also limed, while the watershed of Lohi Lake was not. Furthermore, Hannah flows into Middle, while the historically acidic Clearwater (not limed) feeds Lohi Lake.

Due to chemical recovery from low pH values (<5) to higher pH (>6) over the monitoring period, results from many lakes in the Sudbury region follow the trend of Fig. 2, where toxicity as a result of bound metal is more evident at pH values greater than ~6. The complex interplay among chemical variables needs to be considered when considering trends in F_{TOX}. For several sites in this dataset (Clearwater, Hannah and Lohi) decreased Ni and Cu concentrations (also Zn in Hannah) do not themselves lead to a decrease in F_{TOX} driven by decreased in bound metals. The v_{Ni}, v_{Cu}, and v_{Zn} values actually increased over the period, as a result of decreased competition from H and Al. However, this increase was not enough to offset the decreasing trend in v_H and v_{Al}. Middle Lake was monitored in the spring and summer of 1973 (pH of ~4.6) before being subjected to liming in 1973, the pH was ~6.5 during the latter part of the 1970s, rising to ~7.1 in the early 2000s due to watershed liming. Following initial lime treatment, further recovery in Middle Lake was largely due to lower concentrations of total metals driving lower amounts for modelled bound Ni, Cu and Zn (Cu being the most important). Nelson and Joe Lakes approximately follow the trends of Middle Lake although they had higher initial species richness and lower total metal levels. Whitepine Lake differs from many others in Sudbury as F_{TOX} never exhibits large contributions from bound metals and was not subject to the same degree of chemical changes as other locations. The recovery in F_{TOX} was due to decreased metal and increased DOC concentrations and a ~0.8 pH increase over the 27 years studied. Laundrie and Wavy Lakes remain acidified and have only limited recovery.

3.2.6. Adirondacks lakes region (New York State, United States)

Many of the sites have observed species richness values close to those predicted by the model (Fig. 7). For the region an actual recovery of 0.7 species per decade was observed, whereas the model predicts no change. Thus our modelling suggests that on a regional basis, recovery in Adirondack lakes was not related to changes in chemistry. This and the Sudbury dataset tended to exhibit more sites that had actual species richness values that were closer to the maximum values predicted by the model. Fewer or weaker un-modelled factors were therefore indicated. For example species richness may have been less affected by low dispersal (as indicated in Fig. 5).

3.3. Dietary metal uptake

WHAM-F_{TOX} makes no explicit assumptions about the role of dietary uptake as a vector of toxic metal exposure. Diet may be a significant uptake route, as emphasised by Luoma and Rainbow (2008). Previous work has shown good correlations between metal accumulation by organisms at a variety of trophic levels and WHAM-predicted humic acid metal binding (Tipping et al., 2008, Stockdale et al., 2010 and Tipping and Lofts, 2013). Therefore, it is reasonable to assume that dietary metal uptake by zooplankton will exhibit a similar dependence on water chemistry as direct uptake. We thus assume that trends in both dietary and direct uptake correlate with trends in binding to the HA proxy used for the organism. The validity of this assumption and the effect of the dietary metal uptake is worthy of further study. There may be intervening factors such as relative metal accumulation potential of different phytoplankton species, selectivity of feeding zooplankton to different phytoplankton, digestibility and uptake potential of different metal binding components in the ingested phytoplankton, which may cause deviation from a direct relationship between dissolved speciation and ultimate ecotoxicological effects on local zooplankton.

4. Conclusions

1. The WHAM-F_{TOX} model relates the complex chemical interplay of pH, mixtures of potentially toxic cations, DOC and major ions, with field ecological responses. By using quantile regression and fitting the model on field-derived crustacean zooplankton data responses can be predicted independently of other factors that may influence species richness. The model gives regional predictions for recovery that are

consistent with observations for 3 of the 4 datasets. We have demonstrated that recovery in crustacean zooplankton species richness is both predicted from chemistry and observed across the geographical regions covered in the Bohemian Forest, Norwegian and Sudbury surveys (0.4‑1.6 species per decade).

2. The failure of the model to predict the observed recovery for the Adirondacks region implies that non-chemical factors are responsible for the observed changes.
3. Future work could focus on examining the causes of the differences between observations and predicted maximum species richness. The analysis of chemical toxic effects provided by WHAM-F_{TOX} opens the possibility of identifying presently un-modelled factors, as demonstrated by the relationship with altitude for lakes in the Norwegian survey.

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Tables

Table 1. Mean, 10%ile and 90%ile values of ν_i (the proxy for organism binding, the amount of metal[1] or protons bound per gram of humic acid) obtained from the WHAM modelling for each data set (units of mmol g^{-1} for H and Al, and μmol g^{-1} for Ni, Cu and Zn).

		H			Al			Ni			Cu			Zn		
Survey	*n*	10%ile	Mean	90%ile	10%ile	Mean	90%ile	10%ile	Mean	90%ile	10%ile	Mean	90%ile	10%ile	Mean	90%ile
Norway	231	1.35	1.68	2.17	0.63	4.46	10.6	0.06	0.38	0.95	0.63	4.46	10.64	0.41	4.63	13.18
Sudbury (temporal)	172	1.16	1.56	2.01	3.96	56.0	133	0.43	14.00	36.88	3.96	56.00	133.07	0.54	7.94	16.21
Sudbury (spatial)	116	1.21	1.42	1.68	2.34	16.6	47.4	0.39	7.70	23.24	2.34	16.61	47.45	0.83	7.87	20.28
L. Plessa 107	1	-	4.09	-	-	0.00	-	-	0.00	-	-	0.00	-	-	0.02	-
English Lake District	20	1.12	1.18	1.22	6.02	8.53	10.0	0.17	0.33	0.49	6.02	8.53	10.04	12.24	24.77	46.58
Sweden	80	1.15	1.37	1.65	0.48	3.72	10.6	0.01	0.05	0.11	0.48	3.72	10.60	0.32	1.54	2.44
Adirondacks	431	1.32	1.73	2.33	7.14	22.7	41.5	3.66	15.77	30.32	7.14	22.66	41.53	1.02	11.26	26.62
Bohemian Forest	28	1.12	1.54	1.99	0.19	1.06	2.27	0.00	0.00	0.00	0.19	1.06	2.27	0.24	0.71	1.55
All	1078	1.21	1.62	2.14	1.20	21.1	44.9	0.05	9.45	29.48	1.20	21.15	44.89	0.54	8.19	22.16

[1] Cd data not included as all values are ≤ 0.001 μmol g^{-1}.

Table 2. WHAM-F_{TOX} zooplankton parameters from the best fit using the 90th quantile, with results of the significance testing (p values) for the α_i values and errors (one standard deviation) estimated by bootstrapping.

Parameter	Values	p
$SR_{\mathrm{Czoo\text{-}MAX}}$	12.91 (± 0.50)	-
$F_{\mathrm{TOX\text{-}LT}}$	2.43 (± 0.24)	-
$F_{\mathrm{TOX\text{-}UT}}$	4.64 (± 0.10)	-
α_{H}	1^{a}	< 0.0001
α_{Al}	1.74 (± 0.15)	< 0.0001
α_{Ni}	14.62 (± 2.43)	< 0.0001
α_{Cu}	6.31 (± 0.69)	< 0.0001
α_{Zn}	12.08 (± 2.47)	< 0.0001
α_{Cd}	0.00	n/s
α_{Pb}	34.82	n/s

[a] The α_{H} value is fixed. n/s = not significant

Table 3. Temporal trends in actual and modelled species richness (SR_{Czoo}) assessed using the Regional Kendall test. Values in parentheses show the significance (*p*), the lower the value the smaller the likelihood that there is no trend. Data from L. Plessa 107, English Lake District and Sweden are not included in the temporal analyses due to limited temporal data (1, ≤6 and 5 years, respectively).

Region	Number of sites	Data time span (years)	Change in actual SR_{Czoo} per decade	Change in predicted SR_{Czoo} per decade
Sudbury	9	34	1.40 (<0.0001)	1.57 (<0.0001)
Norway	19	14	0.68 (0.0001)	0.50 (<0.0001)
Adirondacks	35	17	0.74 (<0.0001)	0.03 (0.68)
Bohemian Forest	5	25-26	0.43 (0.0001)	0.52 (<0.0001)

Figure Captions

Figure 1. Relationship between zooplankton species richness and F_{TOX} using quantile regression. The solid line represents the best fit for the 90th quantile. The top left panel shows all data used for the WHAM-F_{TOX} fitting, other panels are included to show how each dataset relates to the overall fit.

Figure 2. Variations in F_{TOX} with pH. The lower horizontal dashed lines represents $F_{\mathrm{TOX\text{-}LT}}$ and the upper dashed line $F_{\mathrm{TOX\text{-}UT}}$. Shown are the 1078 data separated based upon the fraction of F_{TOX} that is comprised of contributions from trace metals (Cu, Ni and Zn). As there will always be some contribution to F_{TOX} from protons we chose to show data where there is at least a 20% contribution from bound trace metals. The Sweden lakes data are show separately as filled triangles.

Figure 3. Temporal trends in field observed species richness (filled circles) and modelled maximum species richness (open circles) based on the WHAM-F_{TOX} results for Bohemian Forest lakes. The horizontal dashed grey line denotes the global maximum (90th quantile) species richness that is predicted by the model. Some years include only observed data as chemistry data was not available.

Figure 4. Temporal trends in field observed species richness (filled circles) and modelled maximum species richness (open circles) based on the WHAM-F_{TOX} results for Norway lakes. The horizontal dashed grey line denotes the global maximum (90th quantile) species richness that is predicted by the model. The lakes designated as highland climate region are designated with (H), lowland sites with (L) and boreal with (B).

Figure 5. Comparison of the mean differences of observed and calculated species richness ($SR_{\mathrm{OBS}} - SR_{\mathrm{MOD}}$), and lake altitude for sites in the Norway study. See Figure 4 for details of which individual lakes are classified as which climate region.

Figure 6. Temporal trends in field observed species richness (filled circles) and modelled maximum species richness (open circles) based on the WHAM-F_{TOX} results for Sudbury lakes . The horizontal dashed grey line denotes the global maximum (90th quantile) species richness that is predicted by the model.

Figure 7. Temporal trends in field observed species richness (filled circles) and modelled maximum species richness (open circles) based on the WHAM-F_{TOX} model results for Adirondacks lakes. The horizontal dashed grey line denotes the global maximum (90th quantile) species richness that is predicted by the model.

Figure 1

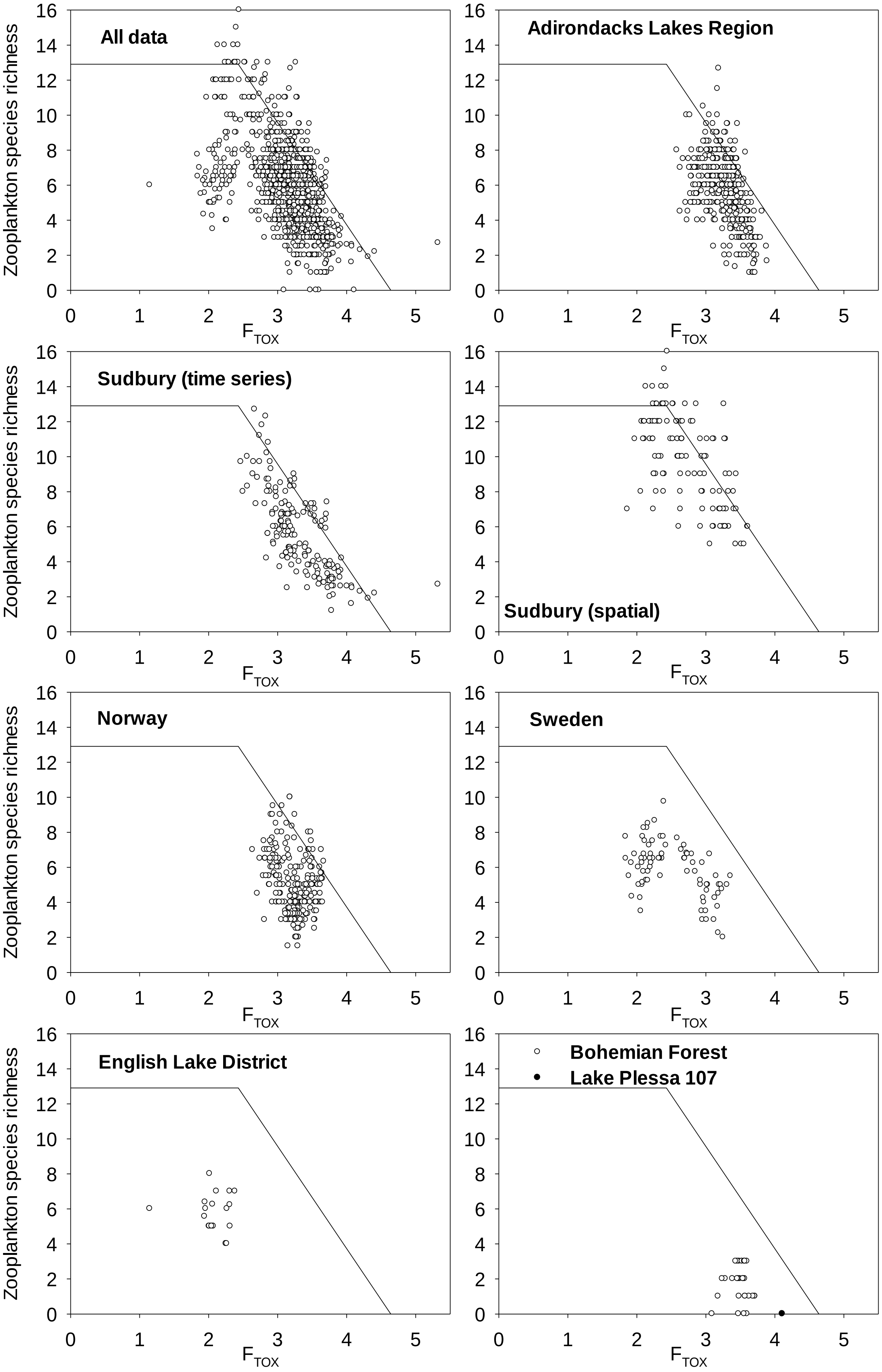

All data
Adirondacks Lakes Region
Sudbury (time series)
Sudbury (spatial)
Norway
Sweden
English Lake District
Bohemian Forest
Lake Plessa 107
Zooplankton species richness
F_{TOX}
0
2
4
6
8
10
12
14
16
0
1
2
3
4
5

Figure 2

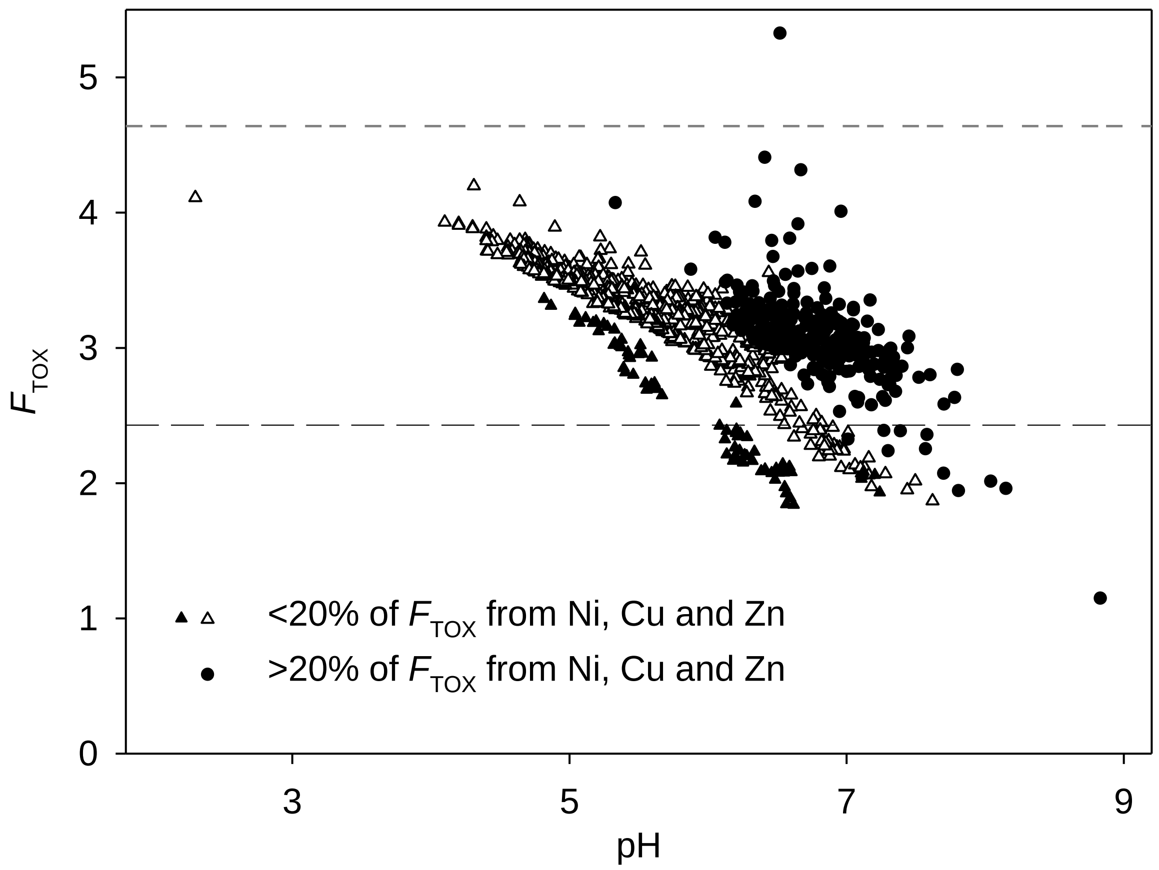

5
4
3
2
1
0
F_{TOX}
3
5
7
9
pH
<20% of F_{TOX} from Ni, Cu and Zn
>20% of F_{TOX} from Ni, Cu and Zn

Figure 3

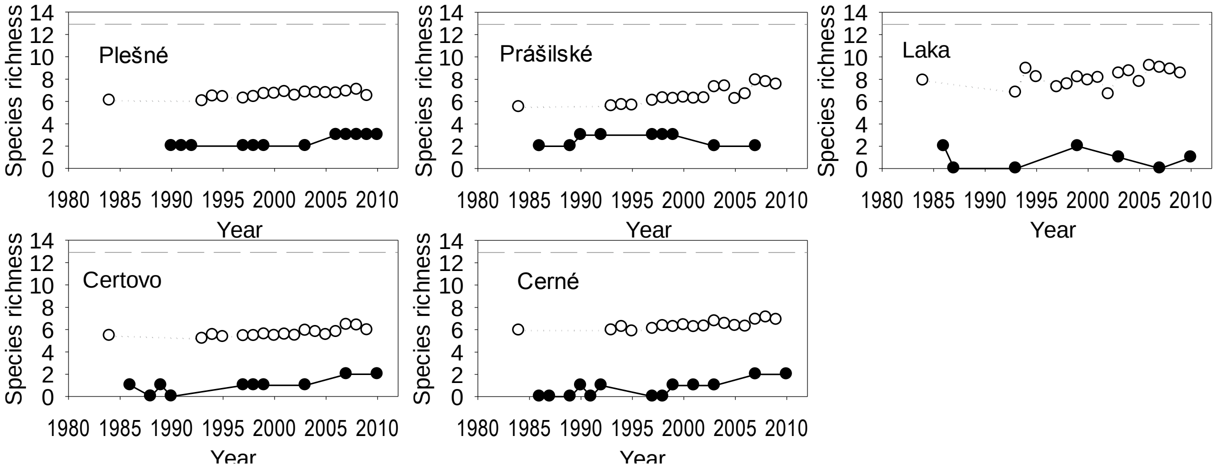

Plešné
Prášilské
Laka
Certovo
Cerné
Species richness
0
2
4
6
8
10
12
14
1980
1985
1990
1995
2000
2005
2010
Year

Figure 4

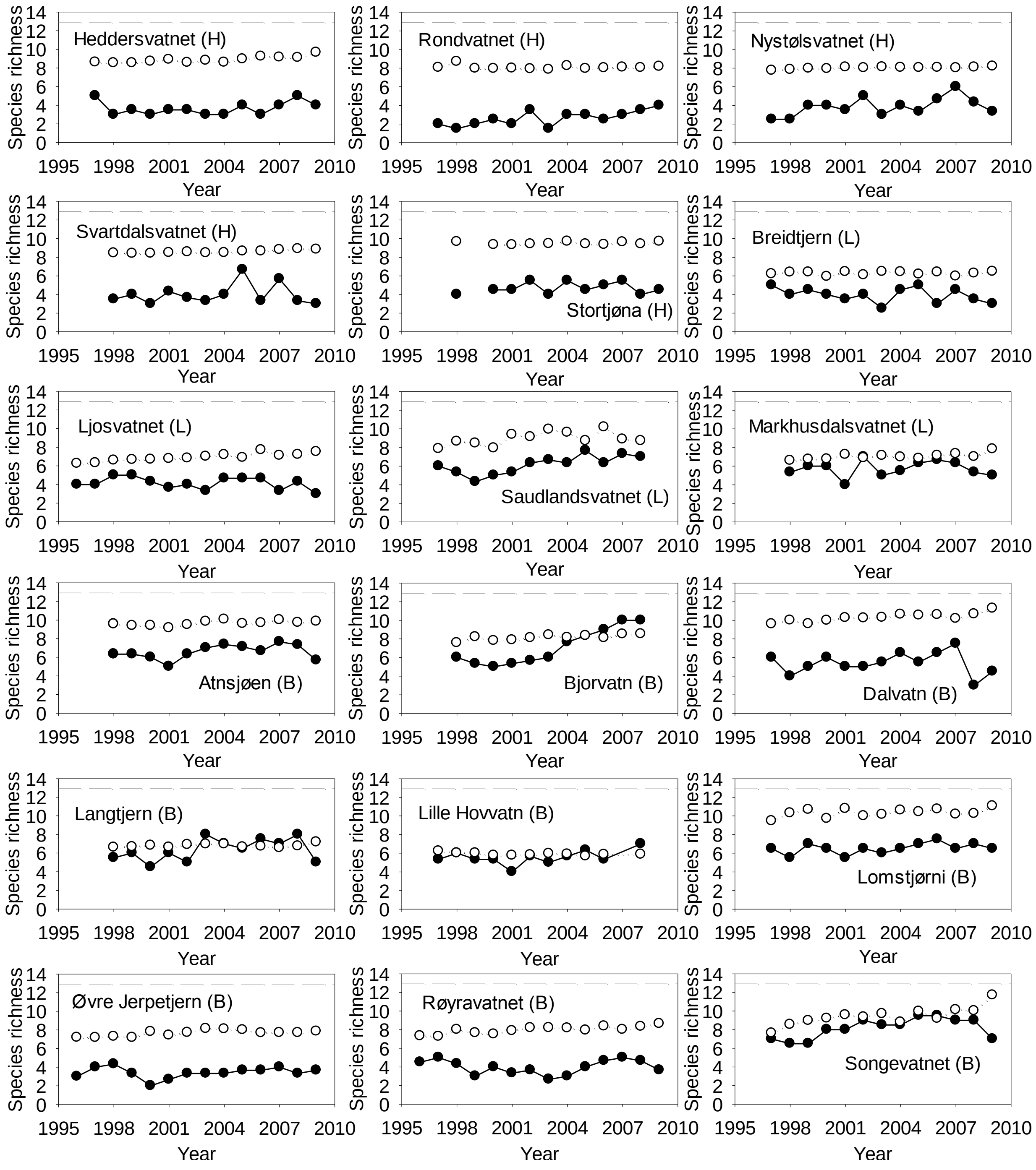
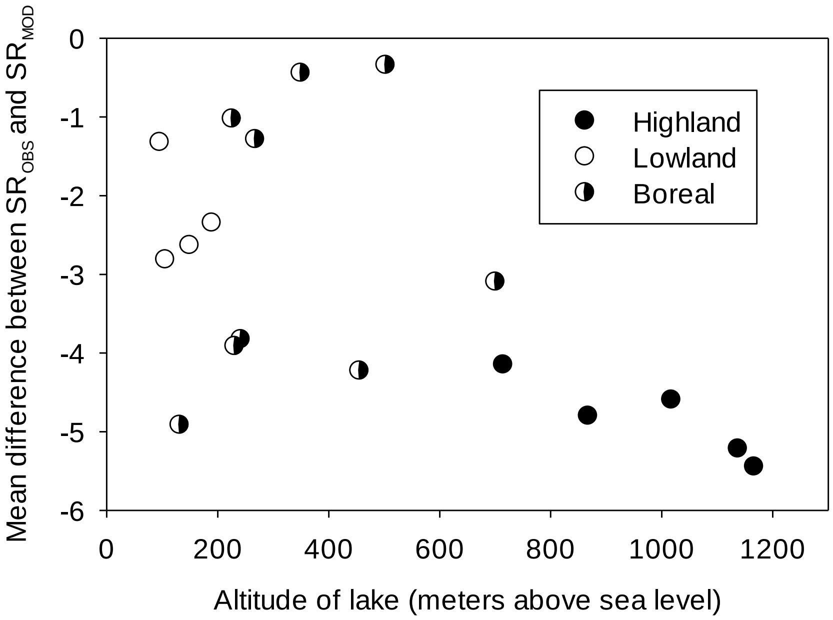
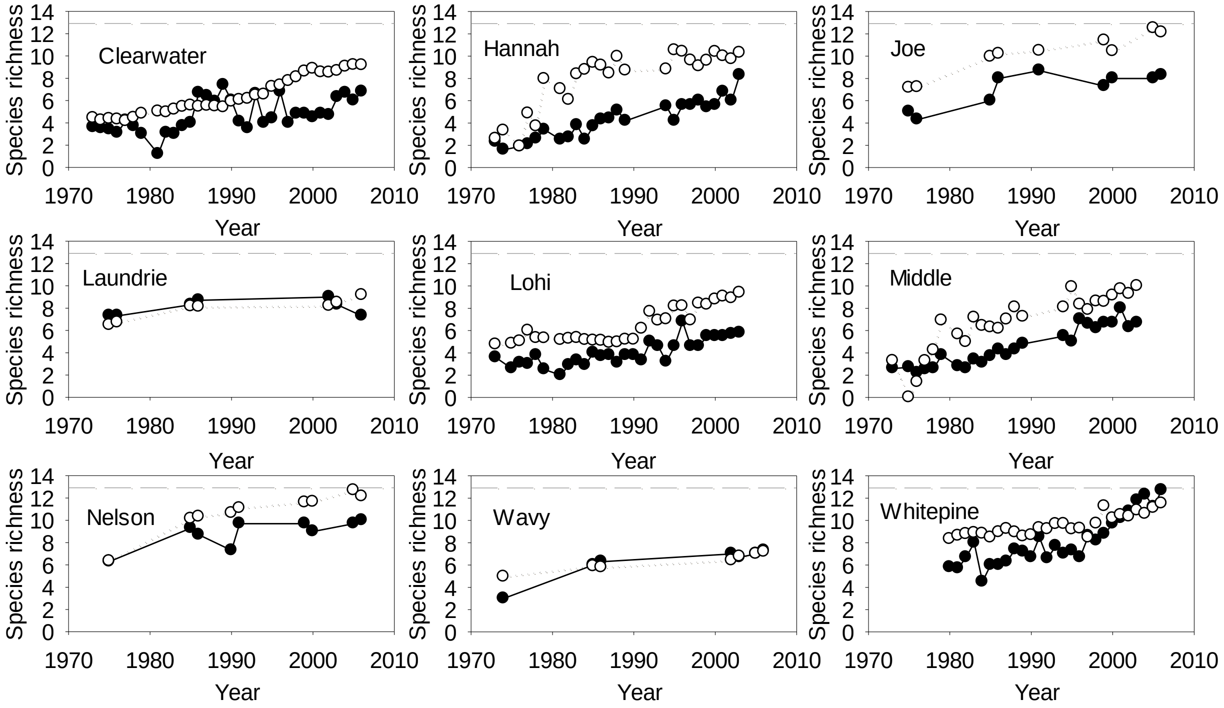

Heddersvatnet (H)
Rondvatnet (H)
Nystølsvatnet (H)
Svartdalsvatnet (H)
Stortjøna (H)
Breidtjern (L)
Ljosvatnet (L)
Saudlandsvatnet (L)
Markhusdalsvatnet (L)
Atnsjøen (B)
Bjorvatn (B)
Dalvatn (B)
Langtjern (B)
Lille Hovvatn (B)
Lomstjørni (B)
Øvre Jerpetjern (B)
Røyravatnet (B)
Songevatnet (B)
Species richness
0
2
4
6
8
10
12
14
Year
1995
1998
2001
2004
2007
2010

Figure 5



Mean difference between SR_{OBS} and SR_{MOD}
0
-1
-2
-3
-4
-5
-6
0
200
400
600
800
1000
1200
Altitude of lake (meters above sea level)
● Highland
○ Lowland
◑ Boreal

Figure 6



Clearwater
Hannah
Joe
Laundrie
Lohi
Middle
Nelson
Wavy
Whitepine
Species richness
0
2
4
6
8
10
12
14
Year
1970
1980
1990
2000
2010

Figure 7 (part 1)

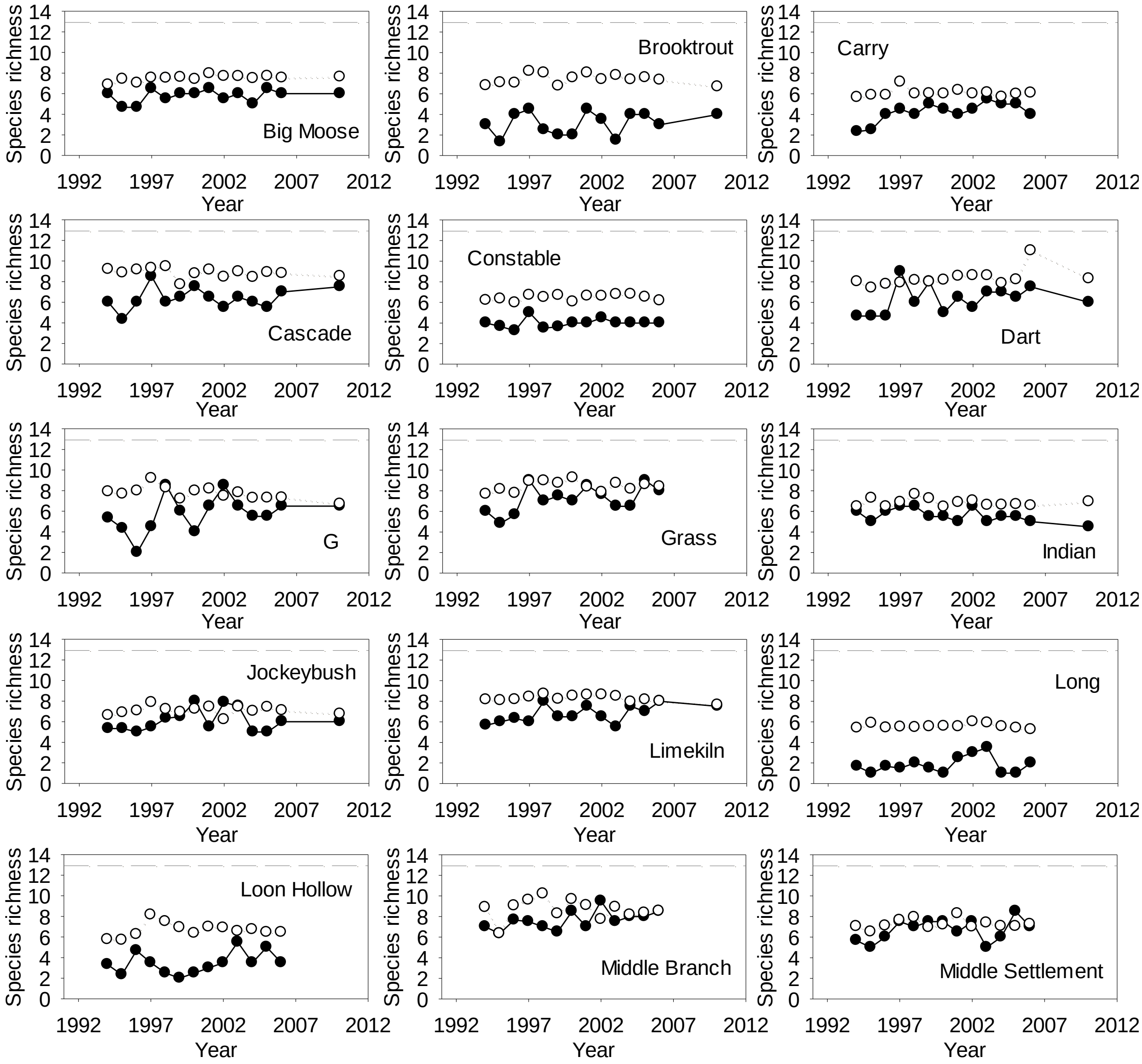


Figure 7 (part 2)